

## **Behavioral Response of Dolphins to Signals Simulating Mid-Frequency Sonar**

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### **LONG-TERM GOALS**

The ultimate goal of the behavioral response study, which includes dolphin and sea lion subjects, is to provide data on the response of marine mammals to the exposure of sonar-like signals across a range of receive levels (dB re 1  $\mu$ Pa). The study is designed to provide data for risk functions used by the United States Navy in predictions of harassment as defined under the Marine Mammal Protection Act.

### **OBJECTIVES**

The objectives of this effort are to:

- 1a) Determine the occurrence and magnitude of behavioral responses observed in bottlenose dolphins exposed to variable levels of mid-frequency (~3 kHz) sounds similar to tactical sonar signals; 1b) apply a scoring analysis to qualitative descriptors of the observed responses; and 1c) calculate a behavioral dose-response function (DRF) for delphinid and otariid species exposed to mid-frequency signals.
- 2) Record heart rate on a subset of the dolphins and sea lions involved in the behavioral response study to determine whether an acute stress response occurs in response to the mid-frequency sound exposure. The response will be related to the exposure characteristics of the sound received by the animal to determine if there is a relationship between the sound exposure and the magnitude and duration of the response.

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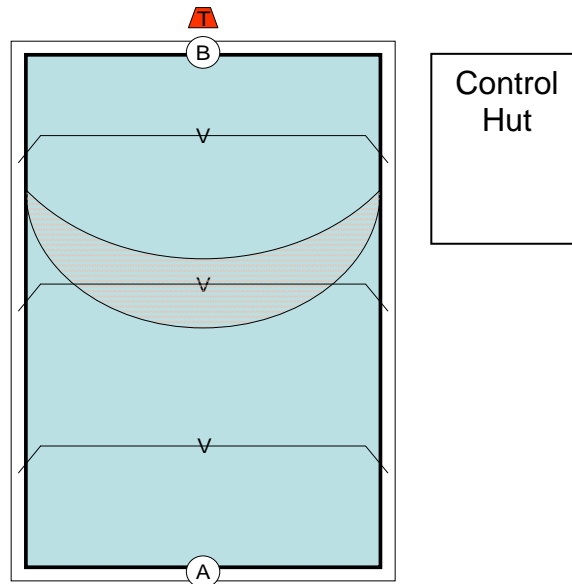
3) Collect blood samples from a subset of the dolphins prior to, immediately following, and for several days/weeks following the sound exposure. Blood collection will be analyzed for cortisol and aldosterone and inspected for parallelism to determine whether a prolonged stress response is triggered by the sound exposure event. In addition, samples will be analyzed for epinephrine to determine if an acute stress response occurs following sound exposure.

## **APPROACH**

A group of dolphins ( $n = 30$ ) and sea lions ( $n = 15$ ) will be trained to a nominal degree of performance on an “A to B” task. The subject will be cued by a trainer to leave a station (A), travel to another station (B) and touch a paddle, and then return to the original station (A) for a fixed fish reward; hereafter termed the ABA task. Subjects will be trained on the ABA task until there is a 100% completion percentage across a 10 trial block. The tasking is relatively easy and a 100% completion percentage is expected for all animals within a few training sessions. Upon meeting the nominal performance criterion, the subjects will perform a 10 trial control session followed by a 10 trial sound exposure session.

Control and exposure sessions will be performed in a 30×60 ft floating pen with an underwater sound projector, designated as T, placed behind station B (Figure 1) and an instrumentation hut placed to the side of the pen. Each trial within the control and exposure sessions will be limited to 30 s. The reward for completing each trial, control or exposure, will be a fixed number of fish. The reward will equate to ~1% of the weight of the daily allotment of fish, which will vary from subject to subject based on body size and dietary needs. For the control session, each subject will perform 10 trials of the ABA task without acoustic exposure. A second session will then be immediately conducted during which the subject will receive an acoustic exposure at a predetermined point along its trajectory within the floating pen. On each trial, the exposure will be performed each time the subject passes the predetermined point on its way to station B. Acoustic exposures will consist of a 0.5 s upward FM sweep (center frequency ~3250 Hz) followed by a 0.5 s CW (~3450 Hz). Subjects will be naïve to the exposure and only one trial block will be used for each subject.

Exposure levels will be consistent for any individual (i.e. all trials within a session will be at the same exposure level), but will be varied from individual to individual in order to provide a range of exposure levels. For dolphin tests, exposure sound pressure levels will range from ~115 to 190 dB re: 1  $\mu$ Pa and will be varied in 15 dB steps. This design permits 5 individual dolphins to be tested at each exposure level. The range of exposure levels and number of subjects per exposure level will be reduced for sea lion tests to accommodate the smaller number of available animals. Dolphins and sea lions will be randomly assigned to an exposure level category for their assigned trial block and the individuals conducting the tests will be kept blind to the assigned exposure levels until the day of the test.



**Figure 1. Layout of the test site for the controlled exposure study. One end of the 30x60 ft pool, station A, is the start and stop point for the marine mammal. On the other end of the pool, at station B, is the paddle that needs to be pushed by the animal. The transducer used for production of the sonar-like stimuli, marked by the red polygon, is approximately 1 m behind station B.**

The floating pen will be outfitted with overhead supports for positioning video cameras such that the entire pen can be monitored. Video recordings of all sessions will be collected and stored to a digital video recorder for later analysis. Known points along the pen will be used to demarcate the point at which sound exposure should occur, i.e. the acoustic exposure will be triggered by an observer when the dolphin or sea lion passes the trigger point.

A trainer will be positioned at station A and another at station B. The session controller, or operator, will be positioned in the control hut and will have direct visual access to the pen as well as video and acoustic surveillance. The trainers and session operator will be in vocal communication via wireless intercoms and communications will be mixed with video signals and stored to DVR. The trainer at station A will be responsible for providing cues to the animal to begin the ABA task and for rewarding the completion of the task. No other interaction with the animal is permitted for the trainer at station A. Both trainers will be responsible for reporting behavioral reactions. The session operator will be responsible for controlling the pace of the session – keeping track of trial times, triggering exposures, directing trial starts and animal recalls.

In addition to video recordings and intercom recordings, monitoring hydrophones will be used to record underwater sounds produced by the animals. Underwater recordings will be mixed down with a duplicate of the video recordings in order to obtain aligned acoustic and visual events. Audio/video files will be used for post-session analysis of behavioral responses.

The suite of behavioral responses anticipated to occur following sound exposure will be *a priori* scored for severity in order to avoid potential bias in severity scoring after data collection. The behavioral responses of individual animals will be recorded during each sound exposure trial (10 exposure trials

in a session) and during each baseline trial. At the completion of the study the behavioral responses will be scored and the results will be used in a four parameter logistic equation to determine the dose-response relationship between sound exposure and the severity and occurrence of behavioral reactions.

Blood samples will be collected approximately one week prior, immediately following the exposure session, and approximately one week after testing. Samples will be analyzed via radioimmunoassay to assess circulating levels of corticosteroid hormones (cortisol and aldosterone) and catecholamines (epinephrine). Levels will be compared between categories and related to exposure level to determine whether acute or chronic stress responses resulted from the exposure. In addition, a subset of the animals that are trained to wear a harness will be fitted with an ECG system to record heart rate during control and experimental trials. As with the catecholamine analysis, variations in heart rate will be assessed for changes associated with the sound exposure.

Dorian Houser will be primarily responsible for conducting the study, overseeing the acoustic and video analysis, performing the RIA analyses, and maintaining the overall project management. James Finneran will contribute with engineering support and acoustic recording setups. Laura Yeates will be responsible for the heart rate recordings of the animals.

## **WORK COMPLETED**

The test site was completed in July of 2009. The site is a floating pier facility and required the addition of electricity for operations to occur. A metal overhead frame was installed at the facility to position overhead cameras. Two gigabit Ethernet machine cameras, one high resolution (1024x1024, 20 fps) and one high speed (640x480, 120 fps), were installed on the overhead frame. Each camera was fitted with a wide angle lens ( $>120^\circ$  fov) permitting complete visual coverage of the 30x60 ft test pen. Bullet cameras were mounted to the control hut and overhead frame permitting higher resolution observation of pen sections. A ceramic, cylindrical ring transducer was installed and has been operated at source levels of 186 dB re 1  $\mu$ Pa (efforts are underway to modify the system to reach the goal source level of 190 dB). Hydrophone monitors and voice over communications have been integrated with the video system for the bullet cameras (captured direct to DVR) and the machine cameras (captured to disk within Norpix software).

Ten bottlenose dolphins have been tested at the time of this report at received levels ranging from 115 to 175 dB. Blood samples were collected from all of the animals and heart rate was recorded from three others.



*Figure 2. Picture of the floating test site. The overhead frame supports two cameras that look down on the 30x60 ft pen. The control hut, from which operations are run, is on the opposite side of the camera frame. Station B, behind which the sound emitting transducer hangs, is the at the mid-point of the far end of the pen.*

## **RESULTS**

Planned analysis of controlled exposure sessions has not yet been completed; it will be performed following the conclusion of the data collection. However, refusal to participate in all of the trials was only observed in one animal that received a 175 dB receive level exposure. Responses at this and lower exposure levels are variable and less pronounced. Conclusions as to the meaningfulness of the results will be withheld until study completion.

## **IMPACT/APPLICATIONS**

The controlled exposure study has the potential to provide the most robust behavioral response data available for use in Navy estimates of impact to marine mammals resulting from mid-frequency signals. The data will be critical to future Navy environmental impact statements and assessments.

## **RELATED PROJECTS**

None.